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Optimal Culture Media development Procedure is Critical for Probiotics Industrial Manufacturing

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ABSTRACT

Probiotics are microorganisms that exhibit a beneficial effect on the host health. Following early development and successful clinical studies, one of the most challenging questions is how to industrially manufacture them in a way to preserve **cells fitness and activity**. Most of the candidate probiotics and particularly Lactobacilli are well-known for their complex growth requirements**. Yeast extracts and peptones (YE)** are widely used in culture media, acting as a natural source of peptides, amino acids and other unique nutrients. In response to the rising demand for high quality and consistent ingredients, **Procelys Applications Labs** have developed bioperformance assays to investigate the importance of nutrients type on **probiotic strains industrial production**.

This work aims at defining an optimal commercial-scale culture medium for ***Lactobacillus acidophilus*** one of the most studied probiotic strain. By repetitive cultures the ability of different YE to enhance the strain growth was evaluated both in term of viability and vitality using a full set of measurements including classical plating and iCinac for acidification kinetics. The specific growth rate of ***L. acidophilus*** was found to be significantly affected by **YE** type provided in the culture medium. Besides the amplitude of difference in yield as compared to classical nitrogen sources was more than 50% in addition with a strong influence on the cells morphology and growth kinetics.

The overall results demonstrate the **careful selection of nitrogen source is key for proper media development** procedure. Moreover, our data indicate the **utilization of suitable YE** and especially **NuCel®786 MG** allows reaching **highly active biomass** while **maintaining cell fitness and robustness**. Additional studies using DOE, orthogonal methods and genome-scale metabolic modelling are ongoing to further explore the basis of the nutrients requirements for this strain.

MATERIALS & METHODS

In this study bacterial growth experiments were first performed at small scale using a microplate reader, shake flasks and then in bioreactors. The viable cells count and different vitality measurements were carried out by flow cytometry as well as classical colonies counting on selected media. Yeast-derived products with specific nutrients composition were added to a final concentration of 20 g/L to the specific culture media of one of the prominent probiotic strain *Lactobacillus acidophilus*. YE samples were autoclaved separately from the sugars, minerals and trace elements solutions 15min at 121°C. Each strain was cultivated at its optimal growth conditions and in a specific culture medium containing the desired growth factors.

Microplates assays were performed in aerobic condition with 400 µL of culture media. The optical density (OD) was automatically monitored at 600 nm. The performance of each yeast-derived product in microplates was evaluated by the maximal OD (ODmax), the maximal growth rate (µmax), and the lag phase length measurement. For culture in iCinac, 250ml flasks containing 200 ml culture medium were inoculated at 1%. pH probes were immersed under sterile conditions and flasks were kept at 37°C water bath. At bioreactors scale several parameters including temperature, pH and electric conductivity were monitored. Cells viability was first quantified by standard plating method and morphology was analyzed by wet mount microscopy.

RESULTS

MICROPLATE EXPERIMENTS

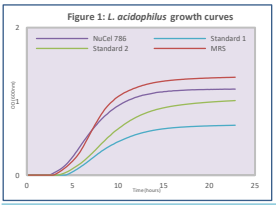


Figure 1. represents the evolution of the OD at 600 nm which can be directly correlated with the microbial growth. The **NuCel 786** showed better results compared to the standards products:

- the final biomass is higher **(+ 16 %** compared to Std 2),
- the maximal growth rate is higher **(+ 46 %)** and
- the lag phase is shorter **(- 14 %).**

(Data not shown, similar results were reproduced in scale up experiments)

SHAKE FLASK EXPERIMENTS

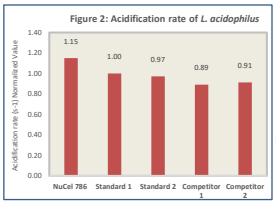
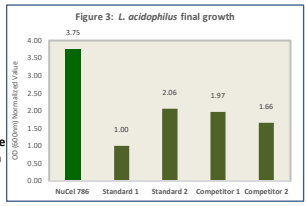


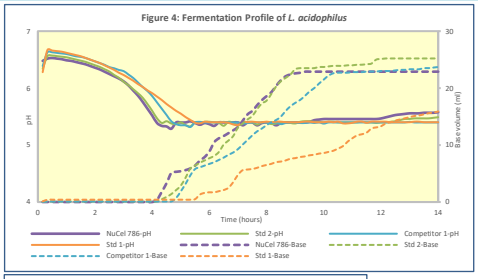
Fig.2 shows the acidification rate of ***L. acidophilus*** determined by iCinac, which monitors pH real time. **NuCel 786** shows a greater acidification rate and higher final OD (Fig.3) compared to standard Y.E and competitor Y.E.

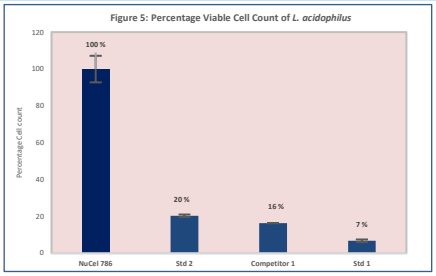
- **+ 18%** higher acidification rate compared to other standard products

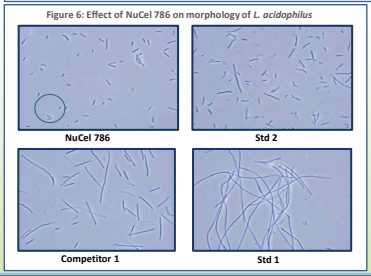
NuCel® 786 tested show a good ability to promote the growth and faster acidification of the probiotic strain *L. acidophilus* at small scale (microplates & shakes flask) as suggested by the growth profiles



BIOREACTORS EXPERIMENTS







Lactobacillus acidophilus viable populations were evaluated using MRS agar plating (Fig:5) and flow cytometry analysis (data not shown). Fig:5 and Fig:6 shows the correlation between quantitative and qualitative analysis of viable cells.

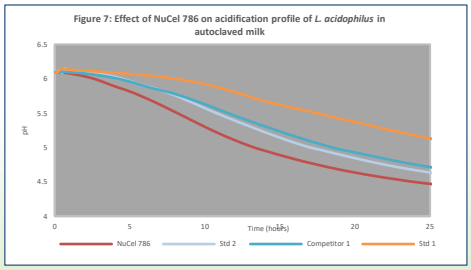
- ✓ **NuCel 786** allows to reach the **highest number of active cells**.
- ✓ Almost 100% viable cells are lost when standard YE is used compared to **NuCel 786**.

Fig:6, shows the morphological state of ***L. acidophilus*** produced with different YE. Results clearly proves with the presences of **NuCel 786**, the cell size distribution shifts to **healthy shorter rods** (highlighted in circle) compared to longer rods with standard YE.

Results demonstrates that **NuCel 786 strongly promotes biomass production, viability & vitality of Probiotic microorganisms.**

Following microplates and shake flasks trials indicating exceptional performance of **NuCel 786**, bioreactor trials were carried out to establish its effects compared with different standard products. Fig.4 shows the **pH and base (NH4OH)** addition monitoring during fermentation, which later can directly linked to **lactic acid production and growth rate**.

With NuCel 786, the growth was over post 8h of fermentation, where as standard 1 imparts slower growth (Prolonged growth post 14h).



After post fermentation harvesting, bacterial culture were inoculated in autoclaved milk to study their vitality (ability to be used post fermentation). Fig:7 confirms the previous results, where NuCel 786 promotes an increase in acidification rate.

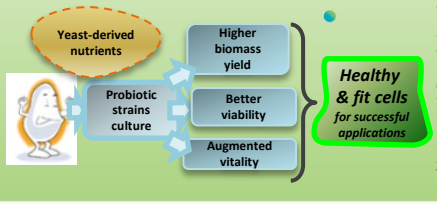
(Further experiments were carried out using ***S.thermophilus*** and ***Bifidobacterium Sp)***

CONCLUSION

The unique and balanced composition of NuCel® range yeast extracts and yeast peptones in complex nutrients such as amino acids, peptides and nucleic acids is key for their **beneficial effect on probiotic strains culture**.

The NuCel® range products specifically NuCel 786 MG designed by Procelys, are the most efficient nitrogen source to ensure robust microbial growth and cell activity maintenance of a wide range of lactic starters and probiotics species.

The aim of this paper was to select the best yeast-derived nutrients, here **NuCel 786** yielding **high biomass level, viability and vitality** which are critical **for probiotics industrial production.** The screening was successfully achieved leveraging **the long term expertise of Procelys Biotech Applications Labs** that are fully committed to assist the probiotic/LAB industry for the accurate selection of performing yeast-derived products.



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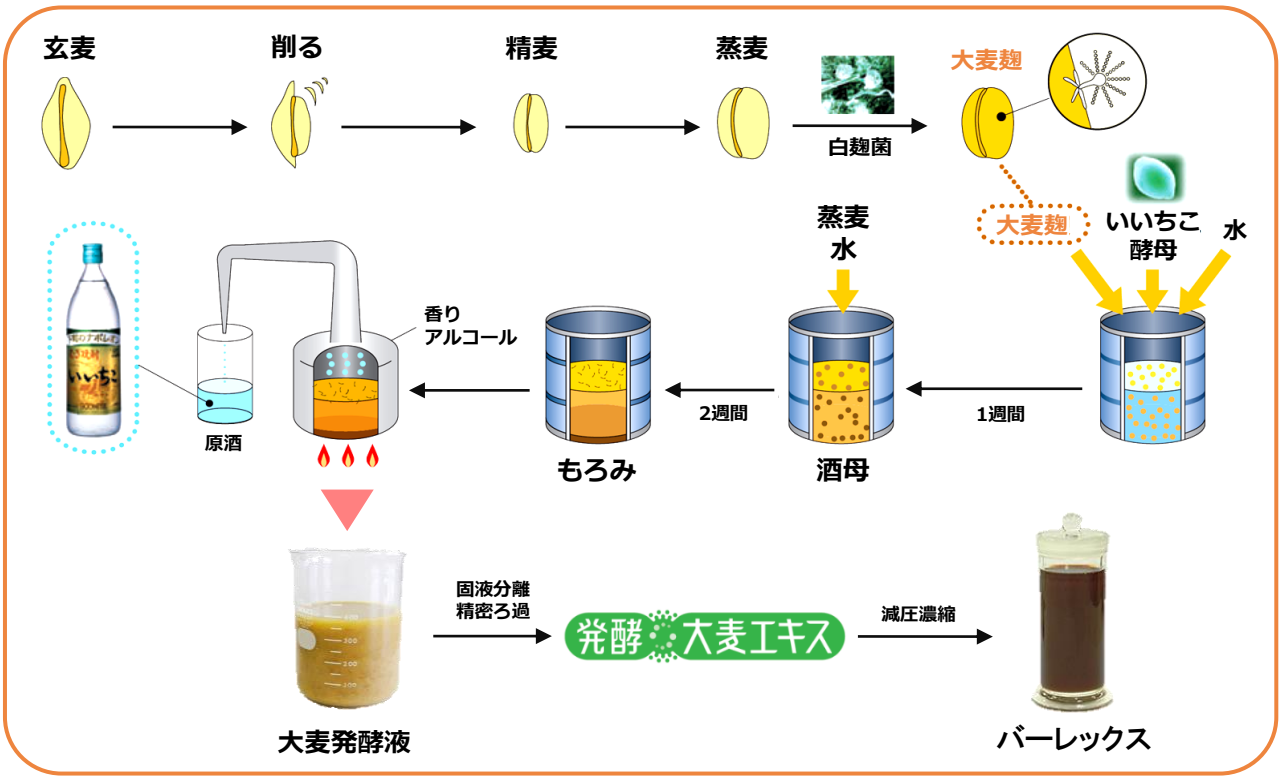
微生物培養基材
バーレックス

『バーレックス』は大麦を起源とするアニマルフリー、Non-GMOの微生物培養基材です。
科学技術に関する技術的専門知識と豊富な実務経験、高い技術者倫理を兼ね備えた
技術士（生物工学）が研究開発しました。
厳格に管理された「いいちこ」の製造工程から得られる大麦発酵液を原料とするため、
窒素源のロット間差が小さく、食品グレードの培養基材としての使用が可能です。
特に乳酸菌の培養において、高い菌体増殖効果を発揮します。

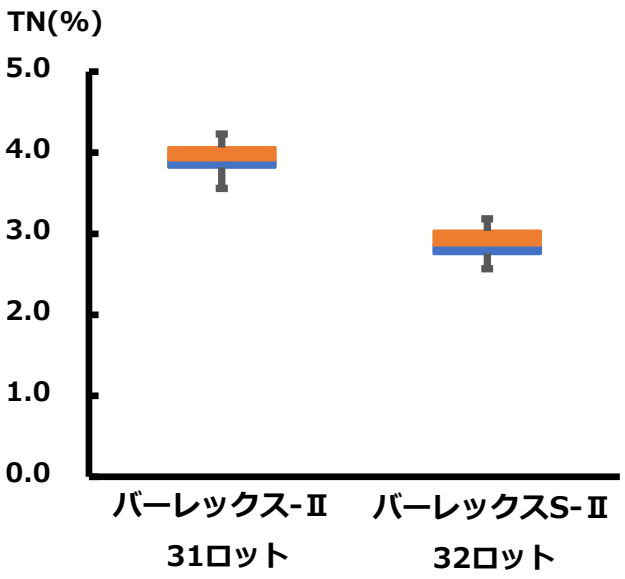
製造工程



培養好適な微生物例

- *Lactobacillus gasseri*
- *Lactobacillus casei*
- *Lactobacillus brevis*
- *Lactobacillus fermentum*
- *Lactobacillus plantarum*
- *Lactobacillus acidophilus* (要オレイン酸)
- *Lactococcus cremoris*
- *Lactococcus lactis*
- *Enterococcus faecalis*
- *Tetragenococcus halophilus*
- *Pediococcus pentosaceus*
- *Bifidobacterium longum*
- *Saccharomyces cerevisiae*
- *Aspergillus kawachii*
- *Aureobasidium pullulans*

安定した品質～全窒素のロット間差～



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